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DNA Polymorphisms in Boreal Owls (*Aegolius funereus*)

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Molecular analyses of polymorphic DNA-fragments are widely used in phylogenetic studies to recognize individuals, to evaluate mating strategies, and to study genetic diversity (Lawless et al. 1997, Primmer and Ellegren 1998). A limiting factor in studies that depend on species-specific variation is the number of available markers. Due to the conservational nature of DNA across species, polymorphic regions that are localized in one species will often be of great use in a number of related species. This is also the case for microsatellites, which are often localized in less conserved areas (Chambers and MacAvoy 2000). The main focus of this work was to establish DNA polymorphism in the Boreal Owl (*Aegolius funereus funereus*) that would be useful for testing paternity, inbreeding, and population genetics. Microsatellites, characterized by short, tandemly-repeated, and highly-polymorphic sequences, were chosen for the analysis. These markers have previously been used for cross-species amplification in birds (Primmer et al. 1996), and in several other species. Although microsatellites are highly polymorphic (varying number of tandemly-repeated motifs), sequences flanking the microsatellite are still conserved enough to be present across related species, and are used for primer binding. As expected, a negative relationship between microsatellite performance and evolutionary distance has been observed (Primmer et al. 1996).

Methods

Blood samples were collected from 44 unrelated free-ranging adult Boreal Owls (Tengmalm's Owl) nesting in Hedmark County, Norway (ca. 61°N, 11°E) in 1998. Natal as well as female breeding dispersal is extensive in the Boreal Owl, causing genetic swamping over large areas (Sonerud et al. 1988). DNA was isolated following standard protocols (Seutin et al. 1991, Krokene et al. 1996) Amplification of microsatellites in Boreal Owl (Table 1) was based on sequences obtained from the Eurasian Eagle-Owl (*Bubo bubo*; Isaksson and Tegelström 2002) Among the microsatellites used in this study Bb111 and Bb126 are GA repeats, whereas, the remaining satellites are CA repeats. Reactions were carried out in 10 μl containing 50 ng of genomic DNA, 0.5 U Taq polymerase, enclosed buffer (Perkin Elmer), 2.5 pmol of each primer and 0.2 mM of each dNTP. Genomic DNA was denatured for 3 min at 94°C prior to amplification. The polymerase chain reaction (PCR-amplification) was run for 35 cycles at 94°C (denaturation) for 15 sec, annealing for 15 sec, and elongation at 72°C for 30 sec. Annealing temperatures varied from 45°C (Bb42) to 48°C (Bb100, Bb101,

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Table 1. Primer sequences (5′–3′) for amplification of Boreal Owl microsatellites. All forward primers (F = forward; R = reverse) were fluorescent labelled. Markers Bb42, Bb100, Bb101, Bb111, Bb126, Bb131, and Bb145 can be found in Isaksson and Tegelström (2002).

MARKER	PRIMER SEQUENCES
Bb20 F	GTGGTGGCACGGCTTGT
Bb20 R	TGTCAAGAGGAAGCATAAAATACAT
Bb120 F	TAATGGTGCTGCTGGTGGAAG
Bb120 R	CATGTGTAGGTGTGGGAGAGAA
Bb134 F	TTTCTCCACGCTTCCTTTTCATA
Bb134 R	AGAAGAATGGCTGGCAAGACTC

and Bb145) to 50°C (Bb111 and Bb134) and 52°C (Bb126). Successful amplification of Bb20, Bb120, and Bb131 was not obtained at any annealing temperatures. Microsatellites were analyzed on an ABI 373 sequencer.

RESULTS AND DISCUSSION

Of the ten primer pairs characterized in eagle-owls, seven successfully amplified DNA from Boreal Owl (Table 2). Five of these were polymorphic in Boreal Owl, whereas, the remaining two were monomorphic within the individuals tested in our analysis. Because Boreal Owls and eagle-owls are among the most distantly related species within the Strigidae family (Mindell et al. 1997), these microsatellites may be of potential use in most species within this family. Our findings could therefore be of great importance for the analysis of population genetics, as well as for parental testing in a wide variety of species within the Strigidae family.

Table 2. Length of alleles, allele frequencies and heterozygosity among 44 unrelated Boreal Owls for seven microsatellites. Microsatellite markers Bb20, Bb120 and Bb131 did not amplify DNA successfully from Boreal Owl.

MICROSATELLITE MARKER	ALLELE LENGTHS	ALLELE FREQUENCIES	OBSERVED HETEROZYGOSITY
Bb42	304 bp	1.000	0
Bb100	296 bp	0.761	0.30
	298 bp	0.239	
Bb101	185 bp	0.477	0.57
	187 bp	0.034	
	189 bp	0.034	
	191 bp	0.455	
Bb111	201 bp	0.023	0.61
	203 bp	0.011	
	205 bp	0.080	
	207 bp	0.625	
	209 bp	0.136	
	211 bp	0.080	
	213 bp	0.045	
Bb126	185 bp	0.989	0.02
	187 bp	0.011	
Bb134	144 bp	1.000	0
Bb145	242 bp	0.898	0.18
	256 bp	0.102	

RESUMEN.—Hemos utilizado los pares de indicadores con base en secuencias del gran búho euroasiático con el fin de ampliar exitosamente siete microsatélites de loci en el búho boreal (*Aegolius funereus funereus*), de los cuales cinco fueron polimorfos. El número de alelos por locus variaron entre dos a siete. La conservación de los microsatélites de loci entre el búho boreal y el gran búho euroasiático indica que las secuencias del gran búho pueden ser útiles en estudios moleculares para la mayoría de especies de la familia strigidae.

[Traducción de César Márquez]

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